AMENDED CLAIMS

[received by the International Bureau on 16 September 2005 (16.09.2005); original claims 1 and 11 amended; remaining claims unchanged (9 pages)]

1. A double-stranded ribonucleic acid (dsRNA), consisting of first and second single RNA strands, having increased effectiveness in inhibiting the expression of a target gene by means of RNA interference, wherein the dsRNA comprises first and second double-stranded ends, wherein at least one double-stranded end is blunt, wherein only one double-stranded end comprises a nucleotide overhang of 1 to 4 unpaired nucleotides, wherein the unpaired nucleotide adjacent to the terminal nucleotide base pair comprises a purine base; and wherein the terminal base pair of the first double-stranded end comprises a guanine-cytosine (G-C) base pair or the four consecutive terminal base pairs of the first double-stranded end comprises at least two G-C base pairs; wherein the terminal base pair of the second double-stranded end comprises a guanine-cytosine (G-C) base pair or the four consecutive terminal base pairs of the second double-stranded end comprises at least two G-C base pairs; excluding the following dsRNAs:

5'- CAGGACCUCGCCGCUGCAGACC-3' (SEQ ID NO: 1)
3'-CGGUCCUGGAGCGGCGACGUCUGG-5' (SEQ ID NO: 2),

5'- GCCUUUGUGGAACUGUACGGCC-3' (SEQ ID NO: 3)
3'-UACGGAAACACCUUGACAUGCCGG-5' (SEQ ID NO: 4),

5'-CUUCUCCGCCUCACACCGCUGCAA-3' (SEQ ID NO: 5)
3'-GAAGAGGCGGAGUGUGGCGACG-5' (SEQ ID NO: 6),

5'- ACGGCUAGCUGUGAAAGGUCC-3' (SEQ ID NO: 13)
3'-AGUGCCGAUCGACACUUUCCAGG-5' (SEQ ID NO: 14),

5'- CAAGGAGCAGGGACAAGUUAC-3' (SEQ ID NO: 15)
3'-AAGUUCCUCGUCCCUGUUCAAUG-5' (SEQ ID NO: 16) and

5'-CACGUACGCGGAAUACUUCGAAA-3' (SEQ ID NO: 17)
3'-GUGCAUGCGCCUUAUGAAGCU-5' (SEQ ID NO: 18).

2. A double-stranded ribonucleic acid (dsRNA), consisting of first and second single RNA strands, having increased effectiveness in inhibiting the expression of a target gene by means of RNA interference, wherein the dsRNA comprises first and second double-stranded ends, wherein both double-stranded ends independently comprise a nucleotide overhang of 1 to 4 unpaired nucleotides, wherein the nucleotide overhang on at least one double-stranded end is 5'-GC-3'; wherein the terminal base pair of the first double-stranded

end comprises a guanine-cytosine (G-C) base pair or the four consecutive terminal base pairs of the first double-stranded end comprises at least two G-C base pairs; wherein the terminal base pair of the second double-stranded end comprises a guanine-cytosine (G-C) base pair or the four consecutive terminal base pairs of the second double-stranded end comprises at least two G-C base pairs; excluding the following dsRNAs:

5'- CCGCUUGACUGCAGAGAGUGC-3'	(SEQ ID NO: 19)
3'-UCGGCGAACUGACGUCUCUCA-5'	(SEQ ID NO: 20),
5'- CAUCUUCUUCAAGGACGACGGC-3'	(SEQ ID NO: 21)
3'-UGGUAGAAGAAGUUCCUGCUGC-5'	(SEQ ID NO: 22),
5'- GGUGGCGCUGGAUGGUAAGCCGC-3'	(SEQ ID NO: 23)
3'-UACCACCGCGACCUACCAUUCGG-5'	(SEQ ID NO: 24),
5'- UCCCCAGGAGGCCUGCGGGAGC-3'	(SEQ ID NO: 25)
3'-GGAGGGGUCCUCCGGACGCCCU-5'	(SEQ ID NO: 26),
5'- UGCAGCUUCGAAGCCUCACAGA-3'	(SEQ ID NO: 27)
3'-CGACGUCGAAGCUUCGGAGUGU-5'	(SEQ ID NO: 28),
5'- UGGGGAGAGAGUUCUGAGGAUU-3'	(SEQ ID NO: 29)
3'-CGACCCCUCUCUCAAGACUCCU-5'	(SEQ ID NO: 30),
5'- ACCUCCGCAACAACUACGCGC-3'	(SEQ ID NO: 31)
3'-GAUGGAGGCGUUGUUGAUGCG-5'	(SEQ ID NO: 32),
5'- GUAGACCUUGCUACUGCCUGC-3'	(SEQ ID NO: 33)
3'-ACCAUCUGGAACGAUGACGGA-5'	(SEQ ID NO: 34),
5'- CAUGACGGAACUAGAGACAGC-3'	(SEQ ID NO: 35)
3'-UGGUACUGCCUUGAUCUCUGU-5'	(SEQ ID NO: 36),
5'- CUCUACGCUUGUACGAGGAGC-3'	(SEQ ID NO: 37)
3'-CAGAGAUGCGAACAUGCUCCU-5'	(SEQ ID NO: 38),
5'- CAGACUUCGGAGUACCUGCGC-3'	(SEQ ID NO: 39)
3'-UUGUCUGAAGCCUCAUGGACG-5'	(SEQ ID NO: 40) and
5'- CAUCUUCUUCAAGGACGACGGC-3'	(SEQ ID NO: 41)
3'- UGGUAGAAGAAGUUCCUGCUGC-5'	(SEQ ID NO: 42).

3. The dsRNA of claim 1 or 2, wherein each nucleotide overhang independently consists of 1 or 2 unpaired nucleotides.

4. The dsRNA of claim 1 or 2, wherein at least half of the unpaired nucleotides comprise a purine base.

5. The dsRNA of claim 1, wherein the unpaired nucleotide adjacent to the terminal nucleotide base pair comprises a guanine (G).

6. The dsRNA of claim 1, wherein the unpaired nucleotide adjacent to the terminal nucleotide base pair comprises an adenine (A) base.

7. The dsRNA of claim 1, wherein the nucleotide overhang consists of the sequence 5'-GC-3'.

8. The dsRNA of claim 2, wherein the unpaired nucleotide adjacent to the terminal nucleotide base pair on the second end comprises a guanine (G) base.

9. The dsRNA of claim 2, wherein the unpaired nucleotide adjacent to the terminal nucleotide base pair on the second double-stranded end comprises an adenine (A) base.

10. The dsRNA of claim 1 or 2, wherein the first single RNA strand is an antisense RNA strand, the second single RNA strand is a sense RNA strand, wherein the antisense RNA strand is complementary to a target gene or a portion thereof.

11. The dsRNA of claim 10, wherein a nucleotide overhang is at the 3'end of the antisense strand.

12. The dsRNA of claim 10, wherein the region of the antisense strand that is complementary to the target gene is 19 to 24 nucleotides in length.

13. The dsRNA of claim 10, wherein the antisense strand is 20 to 28 nucleotides in length.

14. The dsRNA of claim 10, wherein the antisense strand is 21 nucleotides in length.

15. The dsRNA of claim 1 or 2, comprising at least one chemically modified nucleotide.

16. The dsRNA of claim 15, wherein the chemically modified nucleotide comprises a non-natural base.

17. The dsRNA of claim 15, wherein the chemically modified nucleotide comprises a 2' modification.

18. The dsRNA of claim 17, wherein the 2' modification is selected from the group consisting of a 2'-amino modification, a 2'-alkyl modification, and a 2'-O-methyl modification, a 2'-O-ethyl modification, a 2'-O-propyl modification, a 2'-O-allyl modification, a 2'-O-aminoalkyl modification, and a 2'-deoxy-2'-fluoro modification.

19. A method for the targeted selection of a double-stranded ribonucleic acid (dsRNA), consisting of first and second single RNA strands, having increased effectiveness in inhibiting the expression of a target gene by means of RNA interference, comprising the steps of:

(a) selecting a dsRNA comprising first and second double-stranded ends, wherein only one double-stranded end comprises a nucleotide overhang of 1 to 4 unpaired nucleotides in length;

(b) selecting a dsRNA comprising first and second double-stranded ends, wherein the unpaired nucleotide adjacent to the terminal nucleotide base pair comprises a purine base;

(c) selecting a dsRNA comprising first and second double-stranded ends, wherein the terminal base pair of the first double-stranded end comprises a guanine-cytosine (G-C) base pair or the four consecutive terminal base pairs of the first double-stranded end comprises at least two G-C base pairs; wherein the terminal base pair of the second double-stranded end comprises a guanine-cytosine (G-C) base pair or the four consecutive terminal base pairs of the second double-stranded end comprises at least two G-C base pairs; and

(d) excluding the following dsRNAs:

5'- CAGGACCUCGCCGCUGCAGACC-3' (SEQ ID NO: 1)
3'-CGGUCCUGGAGCGGCGACGUCUGG-5' (SEQ ID NO: 2),

5'- GCCUUUGUGGAACUGUACGGCC-3' (SEQ ID NO: 3)
3'-UACGGAAACACCUUGACAUGCCGG-5' (SEQ ID NO: 4),

5'-CUUCUCCGCCUCACACCGCUGCAA-3' (SEQ ID NO: 5)
3'-GAAGAGGCGGAGUGUGGCGACG-5' (SEQ ID NO: 6),

5'- ACGGCUAGCUGUGAAAGGUCC-3' (SEQ ID NO: 13)
3'-AGUGCCGAUCGACACUUUCCAGG-5' (SEQ ID NO: 14),

5'- CAAGGAGCAGGGACAAGUUAC-3' (SEQ ID NO: 15)
3'-AAGUUCCUCGUCCCUGUUCAAUG-5' (SEQ ID NO: 16) and

5'-CACGUACGCGGAAUACUUCGAAA-3' (SEQ ID NO: 17)
3'-GUGCAUGCGCCUUAUGAAGCU-5' (SEQ ID NO: 18).

20. A method for the targeted selection of a double-stranded ribonucleic acid (dsRNA), consisting of first and second single RNA strands, having increased effectiveness in inhibiting the expression of a target gene by means of RNA interference, comprising the steps of:

(a) selecting a dsRNA comprising first and second double-stranded ends, wherein both ends comprise a nucleotide overhang of 1 to 4 unpaired nucleotides in length;

(b) selecting a dsRNA comprising first and second double-stranded ends, wherein the nucleotide overhang on at least one end is 5'-GC-3';

(c) selecting a dsRNA comprising first and second double stranded ends, wherein the terminal base pair of the first double-stranded end comprises a guanine-cytosine (G-C) base pair or the four consecutive terminal base pairs of the first double-stranded end comprises at least two G-C base pairs; wherein the terminal base pair of the second double-stranded end comprises a guanine-cytosine (G-C) base pair or the four consecutive terminal base pairs of the second double-stranded end comprises at least two G-C base pairs; and

(d) excluding the following dsRNAs:

5'- CCGCUUGACUGCAGAGAGUGC-3' (SEQ ID NO: 19)
3'-UCGGCGAACUGACGUCUCUCA-5' (SEQ ID NO: 20),

5'- CAUCUUCUUCAAGGACGACGGC-3'	(SEQ ID NO: 21)	
3'-UGGUAGAAGAAGUUCCUGCUGC-5'	(SEQ ID NO: 22),	
5'- GGUGGCGCUGGAUGGUAAGCCGC-3'	(SEQ ID NO: 23)	
3'-UACCACCGCGACCUACCAUUCGG-5'	(SEQ ID NO: 24),	
5'- UCCCCAGGAGGCCUGCGGGAGC-3'	(SEQ ID NO: 25)	
3'-GGAGGGGUCCUCCGGACGCCCU-5'	(SEQ ID NO: 26),	
5'- UGCAGCUUCGAAGCCUCACAGA-3'	(SEQ ID NO: 27)	
3'-CGACGUCGAAGCUUCGGAGUGU-5'	(SEQ ID NO: 28),	
5'- UGGGGAGAGAGUUCUGAGGAUU-3'	(SEQ ID NO: 29)	
3'-CGACCCCUCUCUCAAGACUCCU-5'	(SEQ ID NO: 30),	
5'- ACCUCCGCAACAACUACGCGC-3'	(SEQ ID NO: 31)	
3'-GAUGGAGGCGUUGUUGAUGCG-5'	(SEQ ID NO: 32),	
5'- GUAGACCUUGCUACUGCCUGC-3'	(SEQ ID NO: 33)	
3'-ACCAUCUGGAACGAUGACGGA-5'	(SEQ ID NO: 34),	
5'- CAUGACGGAACUAGAGACAGC-3'	(SEQ ID NO: 35)	
3'-UGGUACUGCCUUGAUCUCUGU-5'	(SEQ ID NO: 36),	
5'- CUCUACGCUUGUACGAGGAGC-3'	(SEQ ID NO: 37)	
3'-CAGAGAUGCGAACAUGCUCCU-5'	(SEQ ID NO: 38),	
5'- CAGACUUCGGAGUACCUGCGC-3'	(SEQ ID NO: 39)	
3'-UUGUCUGAAGCCUCAUGGACG-5'	(SEQ ID NO: 40) and	
5'- CAUCUUCUUCAAGGACGACGGC-3'	(SEQ ID NO: 41)	
3'- UGGUAGAAGAAGUUCCUGCUGC-5'	(SEQ ID NO: 42).	

21. The method of claim 19 or 20, wherein each nucleotide overhang independently consists of 1 or 2 unpaired nucleotides.

22. The methods of claim 19 or 20, wherein at least half of the unpaired nucleotides comprise a purine base.

23. The method of claim 19, wherein the unpaired nucleotide adjacent to the terminal nucleotide base pair comprises a guanine (G) base.

24. The method of claim 19, wherein the unpaired nucleotide adjacent to the terminal nucleotide base pair comprises an adenine (A) base.

25. The method of claim 19, wherein the nucleotide overhang consists of the sequence 5'-GC-3'.

26. The method of claim 20, wherein the unpaired nucleotide adjacent to the terminal nucleotide base pair comprise a guanine (G) base.

27. The method of claim 20, wherein the unpaired nucleotide adjacent to the terminal nucleotide base pair comprise an adenine (A) base.

28. The method of claim 19 or 20, wherein the first single RNA strand is an antisense RNA strand, the second single RNA strand is a sense RNA strand, wherein the antisense RNA strand is complementary to a target gene or a portion thereof.

29. The method of claim 28, wherein the nucleotide overhang is at the 3'end of the antisense strand.

30. The method of claim 28, wherein the region of the antisense strand that is complementary to the target gene is 19 to 24 nucleotides in length.

31. The method of claim 28, wherein the antisense strand is 20 to 28 nucleotides in length.

32. The method of claim 28, wherein the antisense strand is 21 nucleotides in length.

33. The method of claim 19 or 20, comprising at least one chemically modified nucleotide.

34. The method of claim 33, wherein the chemically modified nucleotide comprises a non-natural base.

35. The methods of claim 33, wherein the chemically modified nucleotide comprises a 2' modification.

36. The method of claim 35, wherein the 2' modification is selected from the group consisting of a 2'-amino modification, a 2'-alkyl modification, and a 2'-O-methyl

modification, a 2'-O-ethyl modification, a 2'-O-propyl modification, a 2'-O-allyl modification, a 2'-O-aminoalkyl modification, and a 2'-deoxy-2'-fluoro modification.

37. A pharmaceutical composition for inhibiting the expression of a target gene by means of RNA interference, comprising: a dsRNA of any one of claims 1-18, or a salt, prodrug or hydrate thereof; and a pharmaceutically acceptable carrier.

38. A method for inhibiting the expression of a target gene in a cell, comprising:

(a) introducing into the cell a dsRNA of any one of claims 1-18, or a salt, prodrug or hydrate thereof; and

(b) maintaining the cell for a time sufficient to obtain degradation of a mRNA transcript of the target gene.

39. The method of claim 38, wherein the cell is a mammalian cell.

40. The method of claim 38 or 39, wherein the target gene is selected from the group consisting of 11-hyroxysteroid dehydrogenase-1, acetyl-CoA-carboxylase-2, acyl CoA:DAG acyltransferase-1, Adenosine A2 receptor, akt, AML-ETO, amyloid beta precursor protein (APP), ApoA1, ApoB, ApoM, APS (adaptor protein with pleckstrin homology and src homology 2 domains, a-synuclein, Aurora A, Aurora B, beta-1 integrin subunit, beta-amyloid converting enzyme (BACE), Bax, beta-catenin, Bcl2, Bcl-XL, Bcr-abl, caspase 8, caspase-3, CCR2, CD40, CD40L, cdk2, chk1, chk2, clotting factor VII, collagen, CD132, CTLA4, cyclin E, Dhcr24, Dipeptidylpeptidase-IV, E-Cadherin, Eg5/KSP, EGF, EGFR1, EWS-Fli1, FAS-fatty acid synthase, FoxA-3, FoxO-1, Fructose-1,6-bisphosphate, Glucose-6-phophate, GM3 synthase, HDAC (histone deacetylase 1-6, 9), Her-2/erb2, HIF1, HMG CoA reductase, hormone sensitive lipase, huntingtin, IKK1, IKK2, LDLR, MDR1, Microsomal Triglyceride Transfer Protein, MMP1, MMP2, MMP9, MyD88, sodium voltage gated type X alpha polypeptide (NaV1.8), NFkB, p38 map kinase mitogen activated protein kinase, p85a regulatory subunit of PI3-kinase, PEPCK, plk1, PTEN, PTP-1B, PU.1, raf, ras, Resistin, SCAP, SERBP-2, SHIP-2, SMAD7, SREBP1C, STAT1, stearoyl-CoA desaturase-1, TERT, TGF-beta-1, TGF-beta-1R1, Topoisomerase I, Topoisomerase II, VEGF, VEGFR1, VEGFR2, VLA1, VLA4, and vanilloid receptor (VR1).

41. A method of treating a disease, malady, or affliction caused by the expression of a target gene in a subject, comprising administering to said subject a pharmaceutical composition of claim 37.

42. The method of claim 41, wherein the subject is a human.

43. The method of claim 41 or 42, wherein the target gene is selected from the group consisting of 11-hyroxysteroid dehydrogenase-1, acetyl-CoA-carboxylase-2, acyl CoA:DAG acyltransferase-1, Adenosine A2 receptor, akt, AML-ETO, amyloid beta precursor protein (APP), ApoA1, ApoB, ApoM, APS (adaptor protein with pleckstrin homology and src homology 2 domains, a-synuclein, Aurora A, Aurora B, beta-1 integrin subunit, beta-amyloid converting enzyme (BACE), Bax, beta-catenin, Bcl2, Bcl-XL, Bcr-abl, caspase 8, caspase-3, CCR2, CD40, CD40L, cdk2, chk1, chk2, clotting factor VII, collagen, CD132, CTLA4, cyclin E, Dhcr24, Dipeptidylpeptidase-IV, E-Cadherin, Eg5/KSP, EGF, EGFR1, EWS-Fli1, FAS-fatty acid synthase, FoxA-3, FoxO-1, Fructose-1,6-bisphosphate, Glucose-6-phophate, GM3 synthase, HDAC (histone deacetylase 1-6, 9), Her-2/erb2, HIF1, HMG CoA reductase, hormone sensitive lipase, huntingtin, IKK1, IKK2, LDLR, MDR1, Microsomal Triglyceride Transfer Protein, MMP1, MMP2, MMP9, MyD88, sodium voltage gated type X alpha polypeptide (NaV1.8), NFkB, p38 map kinase mitogen activated protein kinase, p85a regulatory subunit of PI3-kinase, PEPCK, plk1, PTEN, PTP-1B, PU.1, raf, ras, Resistin, SCAP, SERBP-2, SHIP-2, SMAD7, SREBP1C, STAT1, stearoyl-CoA desaturase-1, TERT, TGF-beta-1, TGF-beta-1R1, Topoisomerase I, Topoisomerase II, VEGF, VEGFR1, VEGFR2, VLA1, VLA4, and vanilloid receptor (VR1).